Exhibit 1

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TRANSGENIC FARM ANIMALS: PROGRESS REPORT

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ABSTRACT

The transfer of genetic material by recombinant DNA technology is an innovative method designed to produce animals with an altered genotype. Transgenic animals may demonstrate a variety of new phenotypes through the expression of the exogenous DNA molecule. Mice developed by these methods have shown that a wide range of promoter elements result in predictable patterns of tissue-specific and hormonally regulated fusion gene products. However, only a limited number of promoter elements have been introduced into domestic farm animals. Although several experiments were initially designed to alter the phenotype through increased rate of growth and improved carcass composition, the lack of specificity and regulation of fusion genes has generally resulted in negative side effects. The commercial sector, however, has invested in this new technology with the goal of producing large amounts of valuable human pharmaceutical drugs in a more efficient manner. If this is to be successfully accomplished transgenic animals must maintain their normal physiological characteristics. The challenge we face is to apply this novel approach to the large domestic species without altering their inherent genetic competence. This report updates the research on transgenic farm animals and outlines a strategy for the production of transgenic goats.

Key words: transgenic, farm animals, goats

INTRODUCTION

A decade ago a new technological breakthrough paved the way for genetically altering the physiology of animals. This achievement involved the direct injection of foreign genes into the first cell of a developing animal (transgenic animals). Transgenic technology has rapidly added to our knowledge of gene regulation and its components. In contrast to the more limited results from tissue culture experiments, transgenics offers

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the advantage of studying the interaction within the entire organism. In addition, the application of this science is focused on improving animal production and utilization. Presently, the major targets include development of disease models, universal donor tissues, generation of high volume biologically active human proteins, and enhancement of disease resistance, growth performance and animal by-products.

Although this technology has been available to the research and corporate communities over the last ten years, relatively little progress has been made in achieving improved farm animal production. However, alternative applications have been identified that can increase the value of the domestic species without effecting classical performance criteria. The area that best exemplifies this approach is in the production of animal bioreactors that can produce and supply the medical community with valuable pharmaceutical drugs in the most economical fashion. This progress report will update the research on genetic engineering of farm animals (Table 1) with emphasis on the production of human pharmaceutical drugs in lactating dairy animals.

PROGRESS IN GROWTH AND DEVELOPMENT

The desire to enhance muscle growth in domestic meat animals has involved the search for growth promoting elements. Two areas have been explored thus far. The first includes peptides such as growth hormone (GH) that effect animal development during the grower phase of a production program. The second involves the differentiation process of muscle cells themselves. Those experiments that involve growth promoting peptides (i.e. GH, GHRH, IGF-1) have been extensively reviewed (1,2,3,4,5) and will not be reiterated in this report.

It should be noted that continuous chronic production of GH in transgenic animals expressing either the heterologous or homologous peptide led to adverse phenotypic changes (2,6).The ability to target expression vectors to muscle tissue would be a more direct and controlled approach to enhance growth performance. For example, myogenic differentiation may be regulated through the alteration of processes that effect muscle fiber development. Preliminary studies of certain viruses that contain the v-ski oncogene suggest that the ski oncogene itself is responsible for the myogenic differentiation process (7). Through the transgenic approach, the chicken ski (c-ski) gene was introduced into mice (8). Three lines of mice were created that expressed the c-ski peptide. Large increases in skeletal muscle were the major phenotypic changes in the animals that expressed the c-ski gene. Hypertrophy of the type II fast fibers was evident through histological analyses. Although the cause and effect of the oncogene on myogenic differentiation is basically unknown, these initial experiments showed that profound changes in muscle tissue can be influenced by the c-ski peptide. It should be emphasized, however, that although the results indicated that expression of an oncogene may specifically effect muscle development and growth, the inability to regulate transgene expression is still apparent. Lack of temporal control leads to the continued synthesis of the oncogene product resulting in a pathologic phenotype. Conceivably this condition could lead to the inability to maintain a mature breeding population.

Table 1. Production of foreign proteins in transgenic farm animals

Species	Promoter	Gene	Fragment Length (Kb)	#Transgenic / #Injected (%)	Body Fluid	Highest Expression Levels	References
Porcine	mMT	hGH	2.6	20/2035 (1.0)	Serum	730 ng/ml	34
	mMT	bGH	2.6	------- -----	Serum	----------	34
Porcine	mMT	hGH	4.0	1/268 (0.4)	-----	----------	35
Porcine	hMT	pGH	2.7	6/423 (1.4)	Serum	~40 ng/ml	36
Porcine	mMLV	rGH	2.6	1/170 (0.6)	Serum	740 ng/ml	6
Ovine	mMT	HSV-TK	8.4	1/155 (0.6)	-----	----------	25
	sBLG	α1AT	11.0	1/49 (2.0)	-----	----------	25
Porcine	mMT	hGRF	2.5	8/2627 (0.3)	Serum	380 pg/ml	2
	mMT	hIGF-I	3.8	4/387 (1.0)	Serum	189 ng/ml	3
Ovine	sBLG	hF1X	11.0	4/307 (1.3)	Milk	25 ng/ml	26
Porcine	bPRL	bGH	2.7	5/289 (1.7)	Serum	20 ng/ml	37
Ovine	mMT	bGH	2.6	2/842 (0.2)	Serum	700 ng/ml	38
	mMT	hGRF	2.5	9/435 (2.0)	Serum	500 pg/ml	38
Ovine	sMT	sGH5	5.26	4/1079 (0.4)	-----	----------	39
	sMT	sGH9	4.86	10/409 (2.4)	Serum	26 μg/ml	39
Porcine	mWAP	mWAP	7.2	5/850 (0.6)	Milk	1 mg/ml	40

Table 1 (cont). Production of foreign proteins in transgenic farm animals

Species	Promoter	Gene	Fragment Length (kb)	#Transgenic / #Injected (%)	Body Fluid	Highest Expression Levels	References
porcine	mMLV	pGH	2.3	6/410 (1.5)	Serum	44 ng/ml	41
	hCMV	pGH	4.7	15/372 (4.0)	Serum	87 ng/ml	41
	mMLV	pGHs	2.8	10/312 (3.2)	-----	----------	41
bovine	mMT	rGH	---	0/24 (0)	-----	----------	42
	mMT	rGH-BE	---	0/34 (0)	-----	----------	42
	mMT	β-gal	---	0/8 (0)	-----	----------	42
porcine	rPEPCK	bGH	---	-----	-----	----------	43
caprine	WAP	htPA	4.0	2/203 (1.0)	Milk	3 μg/ml	27
	β-casein	htPA	15.0	5/295 (1.7)	Milk	3 mg/ml	27
porcine	α,κ	α,κ	----	2/542 (0.4)	Serum	1380 μg/ml	19
ovine	α,κ	α,κ	----	3/222 (1.4)	Serum	----------	19
porcine	κ,γ	κ,γ	14.8	-----	Serum	1 mg/ml	20
ovine	oBLG	ha_1AT	10.6	5/439 (1.1)	Milk	35 mg/ml	29
bovine	ba-casein	hLF	26.0	2/981 (0.2)	------	----------	30
porcine	MSV(LTR)	c-skl	----	29/1091 (2.7)	------	----------	9
porcine	LCR	$a_1a_1B^A$	16.9	3/709 (0.4)	Blood	----------	15

Recently, the c-ski gene was used to create transgenic swine (9). Phenotypic changes were inconsistent among the transgenic animals produced, as demonstrated by varying degrees of hypertrophy involving different muscle groups. In addition, several transgenic pigs showed a myopathic phenotype characterized by muscle fiber vacuoles. Unfortunately, 50% of the transgenic swine experienced weakness in the front and rear legs attributed to muscle atonia.

It is interesting to note that the number of transgenic mice actually expressing the transgene was extremely low (8). Only 7% of the transgenic founder mice expressed the c-ski gene, although Northern analysis was not reported for animals which did not show overt phenotypic change. In comparison, the swine experiments (9) showed that greater than 60% of the transgenic founders expressed the transgene, but only 38% showed phenotypic changes. This may implicate a complication in post transcriptional processing of the gene product or an abnormally modified gene transcript. These experiments reveal considerable lack of knowledge as to the actual effect of the c-ski gene product and its influence on muscle cell development. The differences between the mouse and pig data also emphasize the difficulty of extrapolating between individual mammalian species. Furthermore, the need for an off/on/off switch to control the temporal expression of growth promoting factors is apparent.

PROGRESS IN PRODUCTION OF BLOOD-BORNE PEPTIDES

Blood-borne peptides encoded by heterologous transgenes offer the opportunity to harvest large quantities of product for use in human disease diagnosis and therapy. Secretory proteins can be isolated from the plasma whereas blood cell components can be obtained after lysis of cells. Independent studies have shown that both the human ß-globin gene (10,11) and the human α1-globin gene (12,13) can be expressed in erythroid tissues in transgenic mice. Co-injection experiments with both the human α- and ß-globin gene fused to ß-globin hypersensitive sites also resulted in functional human hemoglobin (14). Interestingly, two additional forms of hemoglobin were produced consisting of mouse/human hybrid molecules. Comparison of the oxygen binding properties of the four dimers showed that the human hemoglobin produced in the transgenic mice had identical oxygen binding properties as native human hemoglobin. The hybrid molecules had intermediate oxygen binding properties when compared to the mouse native hemoglobin.

The mouse model prototypes discussed above were the basis of transgenic farm animal experiments. The goal was to develop a large animal that could produce substantial amounts of functional human hemoglobin which could be harvested from red blood cells for use as a safe blood transfusion substitute. Recently, Swanson and co-workers (15) produced transgenic swine with a fusion gene consisting of four Dnase I hypersensitive sites of the ß-globin linked to two copies of the human α-globin gene and one copy of the human ß-globin gene. Similar to the mouse prototype, a human hemoglobin dimer was produced that represented up to 9% of the total amount of hemoglobin present in blood cells. In addition, the oxygen equilibrium curve for the recombinant human hemoglobin was essentially the same as that of native human hemoglobin. These experiments suggest that large scale production of purified human

hemoglobin can be achieved in transgenic farm animals. However, packaging of the recombinant hemoglobin in artificial cells such as bilayer vesicles or neohemocytes may be required because free hemoglobin does not maintain the same physiologic properties as that found within the blood cell.

The circulatory system is also the target for germ-line encoded antibody production and immunity in transgenic species. This approach involves either the production of specific immunoglobulins designed to increase disease resistance of animals or the generation of animals that can yield large quantities of monoclonal antibodies to be used in diagnostic testing. Presently, few large animal experiments have been reported with this initiative, however, several prototypes have been produced that highlight the efficacy of this goal. In 1983, Brinster and co-workers (16) showed for the first time that transgenic mice could express an exogenous light-chain immunoglobulin κ gene (MOPC-21) in the spleen.

In a similar approach, the human γ1 heavy-chain immunoglobulin gene was introduced into mice (17). The exogenous gene was expressed only in the B lymphocyte lineage of the spleen of transgenic animals. Functionally active hybrid IgG molecules were formed from human heavy-chain components coupled to mouse light-chain components. These results suggested that transgenically engineered IgG molecules could be manufactured in farm animals species. The transgenic animal is derived either through the co-injection of two fusion genes encoding both heavy- and light-chain components or through the cross breeding of two founder generations each producing either the light-or heavy-chain immunoglobulin gene. Such methods have been used to design specific antibodies that may confer immune-mediated protection against domestic farm animals diseases.

Pinkert and co-workers (18) reported the first transgenic mouse model that demonstrated that genomic immunization was feasible. Mice from this experiment expressed genetically engineered antibodies against a bacterial surface antigen, phosphorylcholine. The production of anti-phosphorylcholine antibodies did not require a prior antigenic stimulation in expressing transgenic mice. Also, transgenic animals raised in non-germ-free environments mounted an elevated titer to the pathogen as compared to transgenic animals raised in a germ-free environment.

Recently, expression of a mouse IgA molecule in transgenic mice and pigs underscored the potential of creating farm animal species with germ-line-encoded immunity (19). Fusion genes encoding the mouse α + κ chains for antibodies against phosphorylcholine (PC) were co-injected into pig zygotes. Transgenic pigs were found to express the mouse IgA molecules without inhibiting the endogenous production of Ig. Unfortunately the IgA in the serum did not bind PC. However, monoclonal antibodies produced in transgenic pigs have been shown to have antigenic-binding activity (20). In this study up to 1000 μg MAb/ml was produced indicating that large quantities of specific monoclonal antibodies can be harvested from transgenic farm animals. Further modification of recombinant antibody design should lead to the ability to confer disease resistance and to establish large-scale production of diagnostic antibodies.

PROGRESS IN PRODUCTION OF PROTEINS IN MILK

The mammary gland is a major target for transgenic technology. Genetically derived alterations in milk constituents could lead to an improved food source or to the harvesting of biologically active heterologous proteins. Interestingly, the pioneering work in this area was targeted toward producing a disease model for adenocarcinomas in mammary tissue. The mouse mammary tumor virus promoter element (MMTV LTR) has been fused to several oncogenes resulting in the development of mammary tumors as well as pathology in other tissues (21,22,23). Clearly, expression of active transgene products extraneous to the target tissue may seriously affect the physiology of the animal. Therefore, more restrictive promoter elements have been cloned from milk protein genes such as casein, whey acidic protein and lactoglobulin. These mammary specific promoter elements have been successfully used to generate transgenic animals that produce biologically active peptides from mammary epithelial cells (Table 1). Simons and co-workers (24) used the genomic sheep ß-lactoglobin gene to produce transgenic mice with higher concentrations of sheep ß-lactoglobin than is found in normal sheep milk. This data indicated that with proper genetic engineering of fusion genes in farm animals it would be possible to target foreign protein expression at concentrations that would be competitive with bioreactor methodology.

The pharmaceutical industry is exploring the use of dairy animals to produce genetically engineered heterologous proteins in milk. Sheep carrying fusion genes containing the BLG promoter sequence linked to either human clotting factor IX or human αl -antitrypsin were initially reported in 1988 (25,26). In a recent Biotechnology issue three independent laboratories reported the successful production of transgenic goats, sheep and cattle in order to produce human pharmaceuticals in their milk. Tufts University, School of Veterinary Medicine in collaboration with Genzyme Corporation, Framingham, MA reported the first successful production of transgenic goats (27). These animals were created using an expression vector controlled by the regulatory sequences of the whey acidic protein (WAP) fused to human tissue-type plasminogen activator (tPA) cDNA. The level of tPA produced in the first transgenic goat was only 3 μg/ml. Subsequent transgenic does have produced levels of tPA as high as 3 mg/ml. The tPA was shown to be enzymatically active and was purified 8000 fold with a purity of greater than 98% (28). Analysis of the transgenic protein indicated that it had a significantly different carbohydrate composition from recombinant enzyme produced in C127 cells. This alteration in the structure of the peptide may offer a more stable tPA for human therapy or it may be less effective than the recombinant enzyme produced by C127 cells. Detailed experiments will need to be performed to answer these questions.

In another report found in this issue of Biotechnology, Wright and co-workers (29) working with Pharmaceutical Protein Limited, United Kingdom showed that expression of human alpha-1 antitrypsin (α_1AT) in transgenic sheep could also reach up to 35 mg/ml, a level that is estimated to be commercially viable as an alternative to the cell culture bioreactor system. These expression levels were attributed to the functional ovine ß-lactoglobulin gene promoter fused to the genomic sequences of the human α_1AT. The human α_1AT produced in the transgenic

sheep was indistinguishable from human plasma-derived material from mammalian cell bioreactors. Further biochemical and animal testing is required before the purified protein is considered as a substitute for the human plasma-derived α_1AT.

The ability to maximize the production of a recombinant molecule from milk is apparent from another report in this same issue of Biotechnology. Herman de Boer and collaborators (30) working with Gene Pharming Europe, Netherlands successfully combined "in vitro" fertilization and transgenic technologies to produce dairy cattle with the bovine casein-human lactoferrin coding sequences. Although expression data was not reported, this work underscores the use of abattoir material (oocytes) to produce transgenic cattle. Also, dairy cattle offer the advantage of yielding substantially more milk than can be obtained from either goats or sheep.

A potential caveat to transgenic mammary system targets may be the limitations imposed by the physiology of the gland. Two examples include both caprine and porcine species. The first transgenic goat to produce tPA at levels greater than 1 gram per liter experienced a lactational shutdown that coincided with the presence of a curd-like material in the milk. Overt signs of bacterial mastitis were not found and the animal showed no systemic complications. However, a dramatic decrease in milk production and an increase in glandular nodularity occurred over several days. One of the questions raised by this anomaly was; is enzymatically active tPA soluble at such high concentrations or could it be acting on other milk constituents to cause a precipitate to form? Preliminary experiments showed that degradation of caseins had occurred (P.DiTullio, personal communication). It is also possible that this transgenic founder animal has a mammary pathology independent of the presence of the transgene and tPA production. Female offspring have been derived from this founder and will be studied to answer this question. It should be pointed out, however, that an additional transgenic female has a tPA output of the same magnitude (2-3 mg/ml) as the above female. This second female was milked for 105 days and did not demonstrate the rapid decline in lactation.

Over-expression of the heterologous milk protein, whey acidic protein, in pigs' milk also resulted in a similar lactational shutdown shortly after parturition (31). Histologic examination and explant culture experiments indicated that precocious expression of WAP prior to early pregnancy may have affected mammary epithelial cell development and led to impaired secretory function post-parturition. Although the present research is encouraging, many more constructs are needed to be evaluated before the lactating animal is to be considered as a universal bioreactor for human pharmaceutical drugs.

The first experiments demonstrating the production of a recombinant membrane-associated protein by apocrine secretion into milk of transgenic mice has also been reported (32). The biological activity of the membrane associated cystic fibrosis transmembrane conductance regulator (CFTR) has not yet been established. However, the finding that CFTR can be sequestered into the milk fat globule membrane suggests that other human membrane-associated proteins (e.g. receptors, transporters, and channels)

that incorporate at least in part to the apical cell membrane surface may be produced by this means. The application of this system to CFTR production may have important implications for the development of new therapies for human diseases. The scaling up of the production of membrane associated proteins through larger transgenic animals is presently being explored.

TRANSGENIC GOAT PROGRAM

Estrus Synchronization and Superovulation

Goats used as donor animals were primarily of Alpine or Saanen breeds. The timing of estrus was synchronized with norgestomet ear implants (33). Prostaglandin was administered after the first 7-9 days to remove endogenous sources of progesterone. Thirteen days following progesterone administration, follicle-stimulating hormone was given to goats at a dose of 18 mg over three days in twice daily injections followed by implant removal on d14. During the anestrus season (after February), the dose of FSH was increased to 24 mg. Twenty-four hours following implant removal, the donor animals were mated several times to fertile males over a two-day period. Embryos were flushed from the oviducts on day 2 (d0 equals day of first sign of estrus). The summary of embryo collection over the first four years of the goat program is shown in Table 2.

Table 2: Summary of embryo collection over the four year program

		1989	1990	1991	1992*
#Donors		81	76	68	44
#Ovulations($\bar{x}$)		737(9.1)	607(8.0)	683(10.0)	329(7.5)
#Recovered($\bar{x}$,%)		485(6.0;66)	349(4.6;57)	369(5.4;54)	226(5.1;69)
#Fertilized($\bar{x}$;%)		335(4.1;69)	279(3.7;80)	308(4.5;83)	136(3.1;60)
Stage %	1 cell	91	77	81	70
	2 cell	8	21	17	28
	4 cell	1	1	1	2

*Data represents only the first 2 months of the 1992 season.

Recipient females were mated to vasectomized males to ensure estrous synchrony. Embryos were surgically transferred to the oviducts immediately following microinjection or to the uterus following a 72 hour culture period. The summary of the recipient population is shown in Table 3.

Table 3: Summary of transfer over the four year program

	1989	1990	1991	1992*
#Recipients	46	68	48	28
#Embryo Transferred($\bar{x}$)	250(5.4)	248(3.6)	199(4.1)	85
#Transferred to:				
oviduct	212	206	199	82
uterus**	38	42	0	3
#Pregnant (%)	20(43.5)	40(58.8)	32(66.7)	15(53.6)
#Pregnant: oviduct	15	37	32	15(55.6)
uterus	5	3	--	0(0.0)
#Live Young (%)	30(12.0)	56(22.6)	62(31.2)	21(24.7)
#Live Young (%):				
oviduct	23(10.8)	56(27.2)	62(31.2)	21(25.6)
uterus	7(18.4)	0(0.0)	--	0(0.0)

*Data represents only the first 2 months of the 1992 season.
**Embryos transferred to the uterus were cultured for 72 hours in Ham's F12 + 10% FCS, 5% CO_2 in air.

Detection of Pregnancy

Ultrasonography of recipient females was performed on day 47 and 54 to confirm pregnancy. Those recipients that were confirmed non-pregnant were recycled back into the program. Ultrasound detection is essentially 100 percent accurate. However, the determination of the number of fetuses is estimated by detecting the number of fetuses from both the right and left flanks and dividing by two. Our present method does not accurately determine whether the fetus is in the right or left uterine horn.

Parturition Induction

To ensure that all births are attended by our staff, we induce parturition with prostaglandin on day 145 of pregnancy. Essentially 100 percent of our recipients will deliver on the morning of day 147. The majority of deliveries are termed natural deliveries, although all deliveries are manually assisted. Young are taken immediately from the mother and hand reared using heat treated colostrum and artificial goat or lamb milk replacer. Kid husbandry has changed slightly from one year to another to maximize the growth curves over a 12 week period. Growth curves indicate acceptable growth and development employing our hand-rearing techniques.

DETECTION OF TRANSGENIC KIDS

DNA is extracted from nucleated blood cells as well as from a small ear clipping. Following digestion with restriction enzymes, DNA is fractionated and blotted onto nitrocellulose. Specific probes are used to identify the fusion genes from the corresponding endogenous genes. The summary of the production of transgenic goats over the first four years of the program is shown in Table 4.

Table 4: Production of transgenic animals over the four year program

	1989	1990	1991	1992*
#Kids Born	30	56	62	25
#Kids Transgenic	3	4	3	2
%Transgenic	10.0	7.1	4.8	8.0

*Data represents only the first 2 months of the 1992 season.

Lactation of Transgenic Females

As noted above the lactation profiles of transgenic females may be directly related to the expression vector, the protein produced and the relative protein concentration in the milk. One of our transgenic females expressed tPA at an initial concentration of 9 mg/ml leveling off to 3 mg/ml through the remainder of lactation. Unfortunately, the doe experienced lactational shutdown for reasons that are still under investigation. This female was induced to lactate using a regime of estrogen and progesterone (unpublished data). She yielded a low volume of milk and again proceeded to suspend production. It is interesting to note that the induced lactation did not show the initial 9 mg/ml as was found in the natural lactation. However the levels of tPA were comparable to the 3 mg/ml level post-colostrum. Recently, a second lactating transgenic female secreting tPA at comparable levels did not experience a rapid lactational shutdown. Studies of additional transgenic goats expressing tPA or other biologically active peptides are required to recognize possible limitations to this alternative approach to produce human pharmaceutical peptides.

SUMMARY

The transfer of genetic material by recombinant DNA technology has many applications in the field of medical research. The most prominent areas include laboratory animal disease models and basic research in the regulation of specific genes. The ability to alter the genome of the domestic species has created several new opportunities such as "molecular pharming", heightened productivity and disease resistance of farm animals Current lack of knowledge in multigene interactions has limited progress on transgenic farm animals. The most promising research in single gene integration are studies in mammary gland production of human pharmaceuticals. Continued progress in this area will require a greater understanding of mammary cell physiology and gland development. The potential for transgenic technology to create beneficial changes in our farm animal species still remains speculative but recent investigations are encouraging.

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